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The laboratory rat is an important model for studying breast cancer due to the many similarities in this disease between rats and humans. However, limited knowledge in manipulating the rat genome through transgenesis has prevented researchers from answering important questions in breast cancer research as well as in research on other human cancers. We proposed to carry out a series of detailed studies to optimize the many variables in transgenic manipulation, to extend transgenic rat technology to inbred rat strains, and to develop rat embryo cryopreservation. We have evaluated multiple variables in media composition and microinjection technique, generated multiple transgenic rat lines from several DNAs, all of which should be important for breast cancer research. We have further developed a procedure for rapid and efficient cryopreservation of rat embryos using morulae from superovulated rats that results in our ability to recover about half of frozen embryos as viable pups after transfer into pseudopregnant recipient rats. This procedure can be successfully applied to morulae obtained from rats of outbred Sprague-Dawley or inbred Wistar-Furth strains. In the course of establishing cryopreservation procedures, we have generated banks of frozen morulae from several transgenic rat lines and parental inbred strains that are used in breast cancer research.

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Anne E. Griffo 12/10/97

PI - Signature Date

TABLE OF CONTENTS

Please note: Page 2 (abstract), Pages 8-13 and 17-23 contain unpublished data and therefore should not be distributed.

5. Introduction

5a. General Background

Breast cancer is one of the leading cause of death among women, with one out of every nine women in the United States being predicted to develop this disease during her lifetime. As with all cancers, breast cancer is a disease in which numerous cellular and molecular genetic changes are thought to contribute to the multistaged progression of normal cells to a population of cells with unrestricted growth and metastatic potential. Over the last decade two classes of genes, cellular protooncogenes and tumor suppressor genes, have been identified as genes which play critical roles in regulating cell growth and differentiation. Deregulation of gene expression through chromosomal translocation or mutation in the regulatory elements of the gene, alterations in the activities of these gene products through mutation in the coding regions of the genes, or complete loss of these genes from the chromosome through mutation are considered to be mechanisms contributing to the failure of cells to maintain normal growth characteristics.

Both mice and rats have been extensively used as laboratory animal models in breast cancer research, as well as in cancer research in general. For several reasons, the rat is perhaps the more suitable of the two with respect to a model system for human breast cancer. Whereas a high percentage of breast cancer in the mouse is associated with the integration of the mouse mammary tumor virus (MMTV) into the *int*-1 locus with consequent deregulation of *int*-1 expression, there is no known viral etiology of breast cancer in rats, as in humans (1). Second, the progressive disease that leads to breast cancer in laboratory rats bears striking histological similarity to that seen in human breast cancer (2-4). Third, a high percentage of the resulting rat mammary cancers are hormonally responsive, closely mimicking that seen in human breast cancer. Finally, certain inbred strains of rats show susceptibility to breast cancer whereas others show resistance (5,6). Through genetic crosses between these strains, putative suppressors have been identified (7-9). This genetic susceptibility to breast cancer seen in the rat may bear similarity to the human disease where genetic predisposition is considered to be an important factor (10,11). In part for these reasons, the rat is accepted as the animal model of choice for screening chemopreventive drugs for human breast cancer therapy (2).

Transgenic mice have been widely used in breast cancer research. Mouse models have been developed in which the expression of deregulated *int*-1 (12), c-*myc* (13-16), activated H-*ras* (13, 17), activated c-*neu* (18-20), wild type c-*neu* (21), deregulated growth hormone (22), and deregulated transforming growth factor a (23-25) has occurred in mammary tissue. All cases lead to abnormalities in mammary epithelial cells ranging from epithelial cell hyperproliferation without tumor formation to tumor formation, apparently some being similar to ductal carcinoma in situ which is seen in human breast cancers. The most prevalent genetic alterations in human breast cancers appear to be amplification of the c-*neu* locus (26-28), found in approximately 20% of breast cancers, and mutations of p53 (10,11). Unfortunately, discrepancies between the phenotypes of the several activated-*neu* transgenic mouse models has resulted in the lack of a consensus as to the nature of the activities of the *neu* oncogene in mammary carcinoma in these models. A more promising result was obtained from investigators who analyzed transgenic mice with deregulated expression of the wild-type *neu* proto-oncogene in mammary tissue. These mice developed focal mammary carcinomas, but only after long latency. The loss of p53 function through gene knockout led to only a very low percentage of animals with mammary adenocarcinoma (1 out of 26 p53 null mice) whereas there was a high incidence of malignant lymphomas (20 out of 26). These studies provide the best animal models to date for studying the correlation between disruptions in expression or activities of these cellular

genes and the incidence of mammary carcinoma. However, they may not be truly reflective of the genetics, histopathology, or the progressive nature of human breast cancer.

Considering the depth of knowledge generated by previous studies of breast cancer in the rat and the striking parallels between the rat and human disease, the availability of transgenic rat technology would greatly enhance breast cancer research. Transgenic rats would provide an alternative, and perhaps more suitable, animal model for dissecting the molecular mechanisms of mammary carcinogenesis and testing putative therapeutic agents. In addition to providing good models for breast cancer, the rat has been widely used for biochemical and metabolic studies, owing to its larger size. A large portion of research in neuroanatomy and neurophysiology is based upon the rat. The rat is the animal in which the multistage nature of hepatocarcinogenesis has been established and studied (29). All these areas of research would profit immensely from the availability of transgenic rats.

Recently, the Transgenic Animal Facility at the University of Wisconsin Biotechnology Center developed the capacity to generate transgenic rats, primarily with the encouragement of two university colleagues, Dr. Henry Pitot, an expert in hepatocarcinogenesis, and Dr. Michael Gould, an expert in breast cancer. Through our initial attempts at transgenic rat production, we have successfully generated transgenic rats for each of these cancer researchers. However, the state of transgenic rat technology is rudimentary compared to that for transgenic mice and as such has received only limited use to date. Despite our initial successes, the production of transgenic rats is at present an extremely laborious task. As a consequence of the technical impediments we now encounter, the time and cost for generating transgenic rats is many fold higher than that for the generation of transgenic mice. For many investigators, this high cost is prohibitive. Thus, only with further improvements will this technology be as accessible for the generation of transgenic rats as it has been for the generation of transgenic mice.

Because we foresee a long term and expanding demand for transgenic rats, especially in the breast cancer research field, we propose an investigation designed to optimize transgenic rat production. This proposal to optimize transgenic rat technology was initiated because we believe that significant improvements can be made in both microinjection and embryo transfer techniques which would greatly facilitate transgenic rat technology. These advances should lead to the reduced cost in the production of transgenic rats, and to the capacity to generate transgenic rats in inbred backgrounds. Importantly, during the course of our optimization studies, a series of transgenic rat models for breast cancer research will be generated.

5b. Specific Aims and Statement of Work

Therefore, we proposed this infrastructure enhancement grant to provide a resource to the breast cancer research community for the generation of novel transgenic rat models for breast cancer research. The specific aims we proposed are:

(1) To generate transgenic rat lineages specifically for breast cancer research and to make these transgenic rats readily available to the breast cancer research community at a reasonable cost.

(2) To determine the most efficient technical procedures for the rapid generation of transgenic rat lineages on an outbred genetic background and on inbred genetic backgrounds appropriate for breast cancer research.

(3) To develop efficient procedures for rat embryo cryopreservation.

(4) To develop and maintain the necessary resources and establish procedures for ongoing data sharing and communication amongst transgenic rat laboratories and with breast cancer researchers.

To accomplish these specific aims, we developed a Statement of Work that incorporated aspects of all four specific aims into each of two chronological stages. Stage One dealt with the optimization of technologies for transgenic rat production and cryopreservation using outbred rat strains and Stage Two with optimization for transgenic rat production and embryo cryopreservation using inbred rat strains. The first stage of the Statement of Work, originally designed to cover years 1 and 2 of the grant period, included the following points:

(a) Using MMTV-*neu*^{WT} and MMTV-*neu*^{mut} as test DNAs, optimize variables in microinjection and embryo transfer in the outbred Sprague-Dawley background.

(b) Maintain a small breeding colony of the *neu* transgenic rats (6 lineages) for dissemination to other breast cancer researchers.

(c) Develop embryo cryopreservation for Sprague-Dawley rat embryos. Cryopreserve *neu* transgenic rat lineages.

(d) Solicit requests for DNAs from the breast cancer research community. Have advisory board choose DNAs, judged to be of the greatest potential value to breast cancer research, for microinjection during years 3 and 4.

(e) Develop and make available to transgenic rat and breast cancer research communities specialized information databases.

The second stage of the Statement of Work, originally designed to cover years 3 and 4 of the grant period, included the following points:

(a) Using MMTV-*neu*^{wt} and MMTV-*neu*^{mut} DNAs, adapt the technology for transgenic rat production to the Wistar-Furth inbred rat strain.

(b) Using novel DNA provided by breast cancer researchers, adapt transgenic rat technology to an additional inbred rat genetic background.

(c) Using additional DNAs provided by breast cancer researchers, generate transgenic rat models for breast cancer research in inbred or outbred backgrounds, and complete optimization of microinjection and transfer technologies.

(d) Optimize cryopreservation of inbred rat strain embryos.

(e) Maintain a small breeding colony of rat lines for dissemination to breast cancer researchers.

(f) Maintain electronic information databases for use by the transgenic rat and breast cancer research communities.

This page contains unpublished information.

In this third year of the grant award, we continued efforts to complete Stage One of the project and commenced efforts on Stage Two. As mentioned in last year's report, the efforts of the Transgenic Animal Facility on this project were severely curtailed for nearly one year due to the loss of one key personnel and the temporary leave of the second key personnel, and also by the move of the Transgenic Animal Facility into a new building. These events during the latter part of year one through much of year two left our progress nearly one year delayed. Personnel issues were overcome with the hiring of Dr. Joe Warren and the return to work of Ms. Helmuth. Over the last year, we have now completed our basic optimizations of microinjection of outbred Sprague-Dawley rat embryos (Stage One, Part A), made collaborative arrangements with our first outside breast cancer researcher for injection of a novel DNA into Sprague-Dawley rat embryos (Stage One, Part D), optimized cryopreservation techniques for outbred Sprague-Dawley rat embryos and cryopreserved valuable transgenic rat strains maintained on the Sprague-Dawley background (Stage One, Part C), optimized rat embryo cryopreservation on one inbred rat strain (Stage Two, Part D), begun work on generating new transgenic rat lines on the Sprague-Dawley background for other breast cancer researchers (Stage Two, Part C). The ensuing body of this progress report summarizes our work on the individual aims of this grant over this past year.

6. Body

6a. Statement of Work Stage One, Point A (Specific Aim 1): Using MMTV-*neu*^{wt} and MMTV*neu*^{mut} as test DNAs optimize variables in microinjection and embryo transfer in outbred Sprague-Dawley background: Generation of transgenic rats carrying MMTV-*neu*^{wt} and MMTV-*neu*^{mut} DNAs.

An essential aim of this grant is to generate new valuable transgenic rat strains for breast cancer research. During the first year of this grant, we generated numerous transgenic rat lineages with several DNAs of interest to breast cancer research. These DNAs were as follows: First, MMTV-*neu*^{wt}, which consists of a mouse mammary tumor virus long terminal repeat driving expression of the wild type neu protooncogene. The MMTV promoter sequences have been demonstrated to drive expression of linked genes to the mammary epithelium of transgenic mice (19-21) and, hence, would be expected to do so in the rat as well. The neu oncogene has been shown to be a frequently mutated gene in human breast cancers (26-28). Thus, a rat model where high levels of wild type neu would be expressed should be of value in evaluating the role of this protooncogene in breast cancer. Furthermore, such a rat model could be used in studies to evaluate the role of carcinogenic agents as cofactors in neu-associated breast cancers. The second DNA, called MT-*neu*^{mut} we chose to use is one where a mutated neu oncogene is fused to the mouse metallothionein promoter which is inducible by heavy metals such as zinc (22,23,25). The inducible approach was chosen to express the activated oncogene because of the worry that if expression of a mutated oncogene occurred too early in the life of the rat, stable rat lines would never be derived. The final DNA, called H*ras*-K*ras* consists of the transcriptional control regions of the H-*ras* gene fused to the coding sequences of the K-r*as* gene. Activated H-r*as*, but not K-*ras*, is frequently found in rat mammary carcinomas arising as a consequence of treatment with carcinogens (13,17). This transgene DNA is one of a series of transgenes designed to study the mechanisms whereby this differential activation occurs following carcinogen treatment. Over the course of year two of this grant, these transgenic rat lineages have been under study in the laboratory of Michael Gould in the Human Oncology department at University of Wisconsin Medical School. Although we had intended to maintain a small breeding colony of these rats for other investigators in the

breast cancer research community to use, we have delayed in these efforts until Dr. Gould's lab has completed the initial characterization of these transgenic rats. After Dr. Gould's laboratory has finished their assessment of the effects of deregulated neu expression on mammary carcinoma, we will reestablish a small breeding colony for the purpose of disseminating these animals to other interested investigators.

During the second year of this grant, we also began microinjection experiments to produce transgenic rats expressing high levels of transforming growth factor alpha (TGFα) from the MT promoter. As noted in the general background section, deregulated expression of TGFα in transgenic mice is associated with epithelial hyperplasia and subsequent carcinoma development (23-25). Given the close comparison of breast cancer in rats and humans, it should be of value to determine the potential effects of high TGFα levels on carcinoma development in the rat. Furthermore, with the use of an inducible promoter, these transgenic rats can be used to examine the effects of low-level deregulated expression as well as high level deregulated expression on the mammary gland. The generation of these MT-TGFα animals is in progress at the present time.

During this past year, year three of the grant, we continued microinjections of the MT-TGFα DNA, with the generation of two lines of transgenic rats carrying this DNA. Additionally, we performed additional microinjections with the MMTV-*neu*^{mut} DNA because analysis in Dr. Michael Gould's laboratory of the existing lines of rats showed that transgene expression was optimal in only one of the existing lines. We generated an additional two founder transgenic rats whose germline status is now being determined through breeding experiments. See Table I for a summary of our results on generating transgenic rats with various DNAs.

A goal listed for the second year of this grant was to solicit requests from the breast cancer research community at large for additional DNAs to use in the generation of transgenic rat models for breast cancer research (Statement of Work Stage One, Point D). We had delayed soliciting requests because our progress on the technical aspects of our work was impeded by the long time period of down time between the time Dr. Lohse left and Dr. Warren arrived. With the reestablishment of our transgenic rat program under the direction of Dr. Warren, we felt during year three that our initial optimization studies had been completed. We, therefore, opened solicitation for transgenic rat projects from breast cancer researchers outside of the University of Wisconsin. We have now arranged collaboration with our first of these researchers, Dr. Gail Sonnenshein, Professor of Biochemistry at Boston University, who is studying the role of NF-kB/Rel in mammary tumors. Her initial animal studies were performed using mouse models, but she feels that the rat is a more suitable model for her planned studies. Given that the DNA construct is proven to express as expected in the mouse, we anticipate success in generating valuable rat models for her studies. Injections of this DNA are scheduled to begin presently.

6b. Statement of Work Stage One, Point B (Specific Aim 2): Using MMTV-*neu*^{wt} and MMTV*neu*^{mut} as test DNAs optimize variables in microinjection and embryo transfer in outbred Sprague-Dawley background: Optimize variables in microinjection and embryo transfer technique.

The second aim of our studies is to investigate ways to increase the efficiency and ease of transgenic rat production. During the past year, we continued studies on the use of various media while microinjecting various DNAs (see 6a). We now are satisfied that we've addressed which of the major variations in media composition will provide for the highest transgenic rat production. As the various DNAs listed in Table 1 were microinjected using various media conditions (Table 2), we also note, as expected that the

efficiency can depend on the particular DNA. The reason for this observation is not clear; but such observations are long standing in transgenic mouse production as well. This appears to be a variable that is not possible to control beyond issues of the purity of the DNA, molecular size of the DNA, etc.

6c. Statement of Work Stage One, Point C (Specific Aim 3): Develop efficient methods for cryopreservation of Sprague-Dawley rat embryos.

As mentioned in background, we have established previously efficient methods for the cryopreservation of transgenic mouse embryos. It is clear that adaptation of this technology to the rat will be critical for the long term success of transgenic rat programs in our facility as well as world-wide. From our experience with cryopreserving hamster embryos performed in collaboration with the laboratory of Dr. Barry Bavister at the University of Wisconsin, we could not assume that procedures for efficiently cryopreserving rat embryos would be identical to those used for mouse embryos. When we began our work to develop techniques for efficient cryopreservation of rat embryos, very little data were available in the literature; the only literature sources coming from Japanese groups who performed there studies exclusively with the Wistar strain of rats. (30, 32).

The freezing and thawing process requires that one be able to dehydrate the embryo with a cryoprotectant before freezing and then rehydrate the embryo after freezing without losing viability of the embryo. There are multiple variables that could affect embryo viability. During the first two year of the grant, we began a systematic evaluation of all the steps in the cryopreservation process using the outbred Sprague-Dawley rat as the strain of choice. During this past year, year three, we have completed our studies to evaluate the requirements for retaining high viability of rat morulae during the cryopreservation process. The focus in the first two years was on identifying optimal culture conditions, and freezing and thawing conditions that would support the in vitro developmental capabilities of frozen/thawed Sprague-Dawley embryos and to determine the in vivo developmental capabilities of morulae cryopreserved according to our optimized procedure. Success at the level of in vivo developmental capability of the cryopreserved morulae is critical, because ultimately, the goal of any cryopreservation protocol is to have the ability to regenerate live animals from frozen embryos. For the intitial studies in years one and two, we worked exclusively with morulae collected from naturally ovulating female Sprague-Dawley rats because natural ovulation usually produces the highest quality embryos with the most uniformity in stage. However, there are many advantages to using morulae collected from females superovulated by treatment with FSH and LH. Most notably, the numbers of embryos from any preimplantation stage of development is higher. Secondly, ovulatory patterns are controlled by the investigator rather than the rats' own cycling. Because the investigator controls the ovulatory cycle the numbers of females and males used for generating the embryos is significantly reduced as is the time that the investigator must spend maintaining the colony, checking females for estrus and setting up matings. Combined with the potential for a higher number of embryos per female, superovulation, if successful, represents a large savings in animal costs and investigator time which outweighs the costs of the hormone and osmotic minipumps used for delivery of the drug. Unfortunately, the response of the female to superovulation tends to vary with the genetic background and some strains do not appear to respond at all to such protocols. Therefore, whether superovulation is a valuable tool must be determined for each strain. In the past year, we determined that Sprague-Dawley giving an average of 42 good quality morulae per donor female rat (See Table 3) as compared to an average of 10-15 per female donor under natural ovulation regimens, approximately a three to four-fold increase. The morulae collected from superovulated donors developed in vitro at the same rate as morulae from naturally ovulating females (See Table 4). However, the long-term in vivo developmental

capability of morulae from superovulated females is reduced as compared to that of morulae from naturally ovulating donors (See Table V), by about half. Overall, the benefits of superovulation with FSH and LH outweigh the drawbacks, at least when using Sprague-Dawley rats.

Over the past year, we have cryopreserved rat morulae from Dr. Gould's H*ras*-K*ras* strains. Cryopreserved H*ras*-K*ras* morulae were tested for recovery and percentage of offspring generated from these embryos was similar to that from nontransgenic embryos (See Table VI). At present, a bank of 400 frozen morulae from this strain is maintained in two liquid nitrogen storage tanks in our Facility and one tank in the laboratory of Dr. Griep in the Anatomy Department.

6d. Statement of Work Stage Two, Point D. (Specific Aim 3): Optimize cryopreservation of embryos from inbred rat strains.

It is important to extend our capability to cryopreserve rat morulae beyond the Sprague-Dawley background for several reasons. As the use of inbred rat strains in microinjection experiments increases, so will the need to preserve newly generated transgenic rat strains on these unique backgrounds. Second, inbred rat strains have been used extensively in breast cancer research. As any geneticist is aware, genetic drift and the development of substrains of strains within certain breeding colonies can present problems over the long term. Sometimes, results can not be reproduced from one laboratory to the next if different substrains are used and even within one's own laboratory, results are sometimes irreproducible because stock rats purchased from commercial vendors will change over the course or time, as inbreeding within the colony occurs. Therefore, the ability to cryopreserve inbred rat strains with the desired genetic characteristics is quite important for consistency of results long-term in breast cancer research. Due to these considerations, we began investigations into how suitable our superovulation and cryopreservation procedures would be for use with specific inbred rat strains. Ultimately, we will investigate three strains: Wistar-Furth, Copenhagen, and Wistar-Kyoto. To date, we have completed work with the Wistar-Furth strain and commenced studies on the Copenhagen strain. As shown in Table 3, the mating behavior of Wistar-Furth rats after superovulation is not as robust as that of Sprague-Dawley 46% females plugged as compared to 76% of Sprague-Dawley). Studies demonstrated that this reduced vigor was not related to the superovulation procedure, but is a general characteristic of the Wistar-Furth rat, most likely the males. The numbers of good quality morulae collected from those superovulated Wistar-Furth females that mated was lower than from Sprague-Dawley with an average of 21 good morulae per Wistar-Furth female as compared to 42 from Sprague-Dawley female. While not as good as response, it was clear that superovulation had occurred, as the average numbers of morulae collected from naturally ovulating Wistar-Further females was approximately 7.5. Lastly, morulae collected from superovulated Wistar-Furth rats were cryopreserved using the procedure we developed with morulae from Sprague-Dawley rats. Table 6 shows that the in vivo developmental capability of cryopreserved Wistar-Furth morulae was similar to that of cryopreserved Sprague-Dawley morulae. Thus, the superovulation and cryopreservation procedures we used with Sprague-Dawley rats is suitable for freezing morulae from the Wistar-Furth strain of rat. However, the mating vigor of the Wistar-Furth strain is less than that of the outbred Sprague-Dawley in two regards: first, the mating behavior itself is reduced and second, the numbers of morulae collected per superovulated females is less. However, because the morulae obtained from the superovulated Wistar-Furth rats survive the cryopreservation process just as well as those from the Sprague-Dawley background, because it is more convenient and time and cost efficient in terms of colony usage to use superovulation rather than natural matings, and there is an increased yield of morulae, it is our opinion that use of superovulation when cryopreserving morulae from the Wistar-Furth strain is valuable. At

This page contains unpublished information.

present, therefore, we have cyopreserved a stock of Wistar-Furth fat morulae with the desired genetic characteristics for breast cancer research.

6d. Statement of Work Stage Point E and Stage Two, Point F (Specific Aim 4): Develop and make available to transgenic rat and breast cancer research communities specialized information databases.

The ability to communicate easily, effectively and efficiently with others in transgenic research and breast cancer research is essential in today's rapidly moving scientific world. To this end, many find it useful to communicate using the Internet where bulletin boards are reaching their highest popularity. In the first year of this grant, we joined two "clubs" on the Internet: Rodent Research and Embryo Mail. Over the past year, we have continued to be involved in dialogue through these electronic means and continue to find these services effective vehicles for rapid and informal discussion with our colleagues.

An extensive, up to date, easy to use directory of transgenic animal researchers and breast cancer researchers who use animal models as their primary model system is necessary to facilitate communication between these large groups of investigators. Our original plan was to enlist the assistance of the University of Wisconsin Biotechnology Center's outreach team with this effort. However, the outreach efforts of the Biotechnology Center have been completely reorganized and restricted in the past year. Thus, the individuals who were expected to perform this function are no longer available. Therefore, it has fallen on the hands of the scientists in the Transgenic Animal Facility to accomplish this task, in addition to their efforts in the biological aspects of the project. Thus, progress is slow. We intend in the next year, to generate the directory.

Within the transgenic animal research community, there are several databases, such as the TBASE database operated by the Johns Hopkins University and an NIH-based database, which aim to list most knockout mice and transgenic animals in existence. These groups have elected to make as their first priority accumulating all of the information on the knockout mice. Thus, the effort to accumulate transgenic mouse and especially transgenic rat data is secondary. Accomplishing our task will then enable us to devise a current listing of all transgenic rodent models for breast cancer research as well as all transgenic rat models. This database will be generated during the next year and the information provided to larger transgenic database units in this country at the end of the grant period.

7. Conclusions

During the past year, we have made excellent progress in achieving our goals which are to generate new transgenic rat models for breast cancer research, improve the efficiency and ease with which transgenic rats are produced, to develop effective methods for cryopreservation of rat embryos and to develop the capacity for state of the art communication between the University of Wisconsin Transgenic Animal Facility and the transgenic rat and breast cancer research communities. We have generated additional transgenic rat lineages with DNAs of interest to breast cancer research, MT-TGFα and MMTV-*neu*^{mut} while completing studies on optimization of microinjection techniques. We have begun a collaboration with a breast cancer researcher from Boston University. We have completed the development of a rat embryo cryopreservation protocol and established that morulae collected from superovulated donor females are suitable for the procedure, thus greatly facilitating the process while saving time and money. We have cryopreserved transgenic rat lines important to breast cancer research, those carrying a *ras* oncogene. And

we have determined that the superovulation and cryopreservation regimen developed for use with outbred Sprague-Dawley rats can be used at least with the Wistar-Furth inbred strain. In so doing, we have cryopreserved a stock of Wistar-Furth morulae with he genetic characteristics desired in breast cancer research.

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9. Appendix

Table 1: Comparison of transgenic rates of various constructs

Table 2: Comparison of transgenic rates in various injection media

Table 3: Efficiency of FSH/LH superovulation on Sprague-Dawley and Wistar-Furth rats

Table 4: In vitro development of superovulated and naturally ovulated morulae

Table 5: In vivo development of unmanipulated morulae after transfer into pseudopregnant recipients

Table 6: Live births from frozen-thawed embryos on inbred and outbred backgrounds

This page contains unpublished information.
Anne E. Griep, PI

Table 1. Comparison of transgenic rates of various constructs

Construct	No. Embryos Transferred	No. Recipients	No. Live Offspring (%)	No. Transgenics (%)
Hras - Kras	2686	65	61	5 (8.2%)
MMTV - NeuN	2858	82	79	7 (8.9%)
MMTV - NeuT	1224	33	16	1 (6.3%)
MT - TGFα	1539	52	68	2 (3.0%)

Hras - Kras - transcriptional control regions of Hras fused to coding sequences of Kras gene

MMTV - NeuN - MMTV long terminal repeats fused to mutated neu proto-oncogene

MMTV - NeuT - MMTV long terminal repeats fused to wild type neu proto-oncogene

MT - TGFα - mouse metallothionein-I promoter fused to transforming growth factor alpha

Table 2. Comparison of transgenic rates in various injection media

Medium	No. Embryos Transferred	Recipients	No. Pregnant Recipients (%)	No. Live Offspring (%)	Transgenics (%)
M2	2319	115	80 (70%)	109 (4.7%)	13 (11.9%)
NG	759	24	19 (79%)	41 (5.4%)	7 (17.1%)
NP	714	16	9 (56%)	25 (3.5%)	1 (4%)
NPNG	586	17	13 (76%)	20 (2.9%)	1 (5%)
DPM2	750	12	9 (75%)	18 (2.4%)	1 (5.6%)
DPNG	229	5	5 (100%)	8 (3.5%)	1 (12.5%)
NG cyto-B	1067	35	23 (66%)	22 (2.1%)	1 (4.5%)

M2 - mouse M2
NG - mouse M2 lacking glucose
NP - mouse M2 lacking phosphate
NPNG - mouse M2 lacking glucose and phosphate
DM2 - mouse M2 containing glucosamine with reduced glucose
DPM2 - embryos presoaked in DM2 and then microinjected in M2
DPNG - embryos presoaked in DM2 and then microinjected in M2 lacking glucose
NG cyto-B - mouse M2 lacking glucose and containing cytochalasin B

This page contains unpublished information
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Table 3. Efficiency of FSH/LH superovulation on Sprague-Dawley and Wistar-Furth rats

Strain	#females given FSH/LH	#Females plugged (%)	Total # embryos collected	#Good morulae (% of total)	Av. # good morulae per female that plugged
Sprague-Dawley	64	50 (78)	3457	2110 (61)	42
Wiistar-Furth	56	26 (46)	1048	551 (53)	21

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Table 4. In vitro development of morulae from naturally ovulated and superovulated Sprague-Dawley female rats

Ovulation regimen	# embryos cultured	# blastocysts at 42 hr (%)
NOM^{+}	42	42(100)[a]
SOM^{++}	90	86 (96)[a]

$^{+}$NOM=Morulae collected from 3 naturally ovulated females

$^{++}$SOM=Morulae collected from 2 superovulated females.

[a]not significantly different ($p>0.05$)

Table 5. In vivo development of unmanipulated morulae after transfer into pseudopregnant recipients

Morulae Transferred	No. Embryos Transferred	No. Recipients	No. Pregnant Recipients (%)	No. Offspring (%)
Unmanipulated NOM into day 4 recipients	43	3	2 (66.7%)	11 (39.3%)[a]
Unmanipulated NOM into day 3 recipients	54	4	4 (100%)	42 (77.8%)[b]
Unmanipulated SOM into day 3 recipients	70	5	5 (100%)	30 (42.9%)[a]

NOM=Morulae collected from naturally ovulated females; SOM=Morulae collected from superovulated females.

[a,b]Values with different superscripts within each column indicate significant differences ($p<0.05$).

Table 6. Live births from frozen-thawed embryos on inbred and outbred backgrounds

Morulae Transferred	No. Embryos Transferred	No. Recipients	No. Pregnant Recipients (%)	No. Offspring (%)*
Frozen-thawed Sprague Dawley SOM	231	14	13 (92.9%)	71 (32.1%)
Frozen-thawed Hras-Kras (on SD bkgd) SOM	106	7	7 (100%)	40 (37.7%)
Frozen-thawed Wistar Furth SOM	61	4	4 (100%)	20 (32.8%)

SOM=Morulae collected from superovulated females.

*Percentages based on the number of embryos transferred into females that established pregnancy.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

1 JUN 2001

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Reports to be changed to "Approved for public release; distribution unlimited"

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